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10 min LC-MSMS analysis of fatty acids in triacylglycerols to compare human serum and food.

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1. Introduction

Triacylglycerols (TAGs) are a type of fat for the most found in fat tissue, but some circulate in the blood. They are a major source of energy for the body. But in excess amounts they may increase the risk to develop atherosclerosis, heart disease and stroke.

A triacylglycerol consists of an esterified glycerol bounded to 3 fatty acids. There are many kinds of triacylglycerols due to the distribution of fatty acids. In function of saturation and unsaturation of fatty acids some are essential for the good health and other are unhealthy.

That's why a multiple reaction monitoring (MRM) method have been set up to screen the different TAGs and their fatty acids distribution in human serum and food.

2. Methods and Materials

2-1. Analytical Method

The method was developed on LC-MS system consisted of a liquid chromatography Nexera LC40 and a triple quadrupole mass spectrometer LCMS-8060 mass spectrometer (Shimadzu Corp.). This method allows the screening of around 150 Triacylglycerols in 10 min with a dwell time between 1 and 3 msec. The analytical conditions used are the following (Figure1).

Chromatographic conditions (LC-40)

Column	:	Shim-pack Velox C18
Temperature	:	50 °C
Mobile Phase A	:	Water + 20 mM ammonium formate
Mobile Phase B	:	80/20 2-Propanol / Acetonitrile
Flow Rate	:	400 µL / min.
Analysis time	:	10 min.
Rinse and injection Solvent	:	80/20 2-Propanol / Acetonitrirle
Injection Volume	:	3 µL

MS conditions (LCMS-8060)

Ionization	:	ESI, Positive/Negative
Nebulizing Gas Flow	:	3.0 L/min
Drying Gas Flow	:	10.0 L/min.
Heating Gas Flow	:	10.0 L/min.
DL Temp.	:	250 °C
Block Heater Temp.	:	400 °C
Interface Temp.	:	150 °C
CID Gas Pressure	:	270 kPa

Figure 1 Analytical Condition

2. Methods and Materials

2-2. Sample preparation

20µL of a pooled of normal human serum from Kojin Bio Japan and 50 mg of the mackerel, were extracted by chloroform and methanol as shown in Figure2.

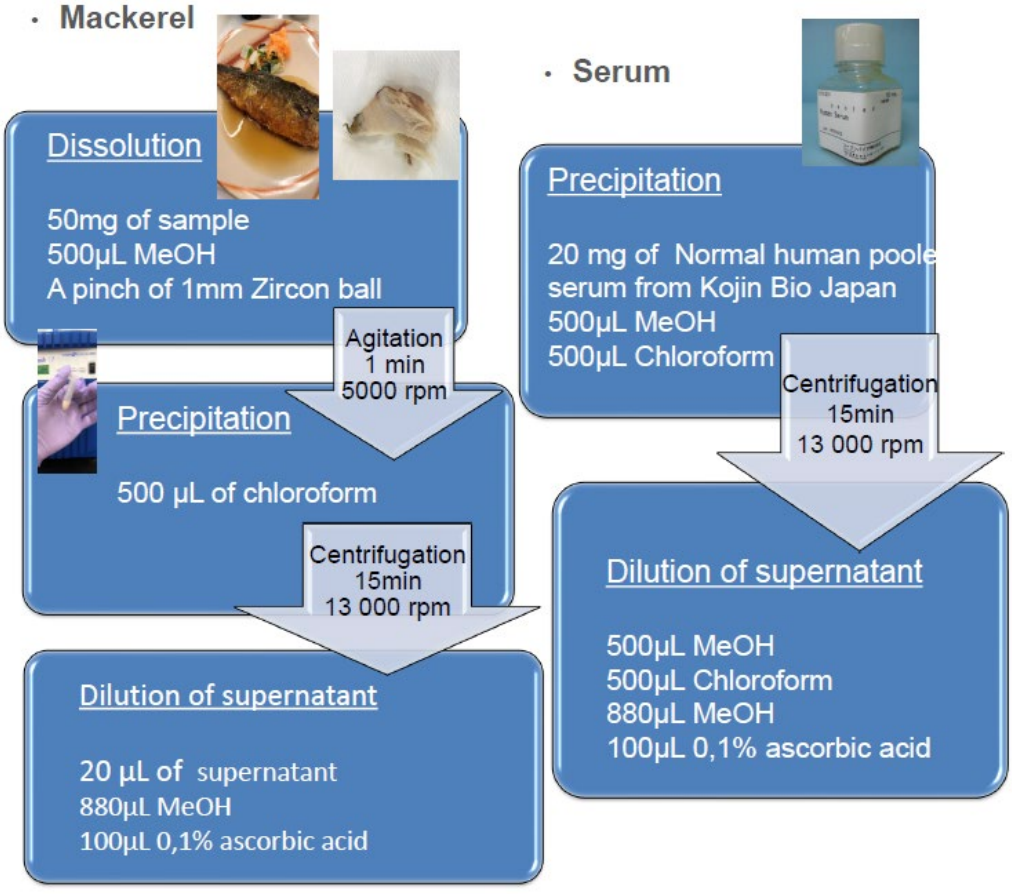

Figure 2 Sample preparation

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3. Results

3-1. Chromatographic profile

A method to screen the fatty acids distribution in 150 different triacylglycerols was developed. The MRM transitions have been defined in order to set up a method which makes it possible to visualize the fatty acids present in triacylglycerols. For this, each transition is composed of a precursor corresponding to the *m/z* of the targeted triacylglycerols and the fragment to the *m/z* of this triacylglycerols minus the value of the fatty acid. To confirm their belonging to triacylglycerols it is necessary that the 3 transitions generate a peak at the same retention time.
This method monitors 450 MRM in 10 min with a good separation of isotopes (Figure3). And the carry-over is evaluated at less than 1%.

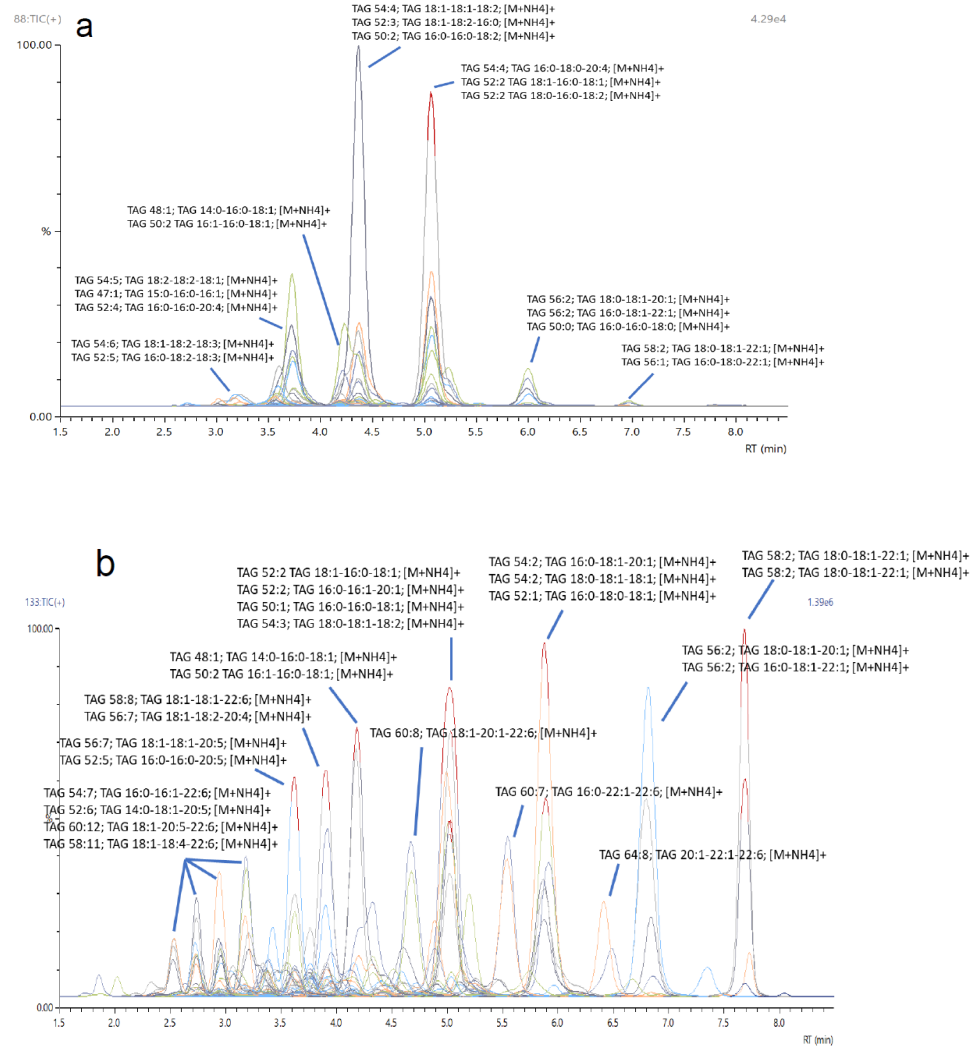
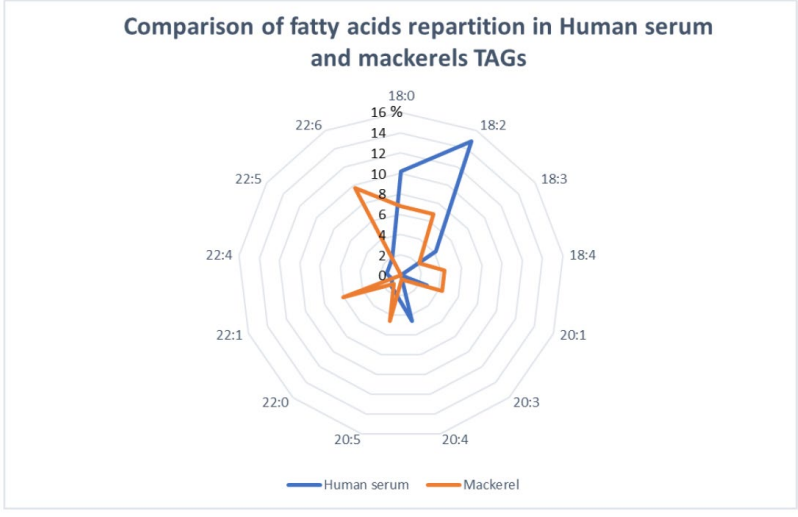


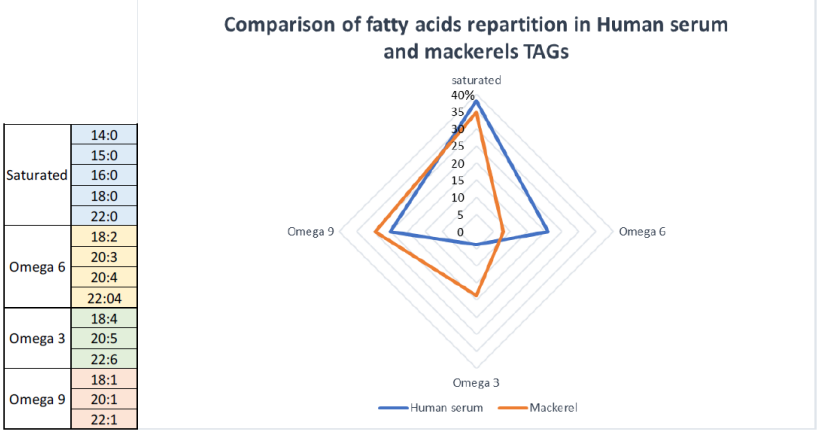
Figure 3 Chromatographic profiles for serum(a) and mackerel(b)

3. Results

3-2. Qualitative comparison between serum and mackerel

The first results showed that unlike human serum, the mackerel contains lot of different kind of triacylglycerols. Among the 104 triacylglycerols detected in both samples 35 Triacylglycerols are common between human serum and mackerel. 51 Triacylglycerols were found in more in mackerel and only 18 in human serum(Figure 5).
In serum, the triacylglycerols mainly contain arachidonic (20:4), linoleic (18:2) and stearicacid (18:0). In contrast, the mackerel mainly have docosahexaenoic(22:6), eicosapentaenoic (20:5), stearidonic (18:4), erucic (22:1) and eicosenoic acid (20:1).
The serum analyzed mostly contains omega 6 and saturated fatty acids and mackerel contains lot of omega 3 and 9 (Figure4).





Saturated	14:0
	15:0
	16:0
	18:0
	22:0
Omega 6	18:2
	20:3
	20:4
	22:04
Omega 3	18:4
	20:5
	22:6
Omega 9	18:1
	20:1
	22:1

Figure 4 Serum and mackerel TAGs and fatty acids distribution

4. Conclusions

This method allowed to screen 150 triacylglycerols and to know the fatty acids composition in serum and food sample. The first use of this method, on human serum and mackerel demonstrated a similarity on 35 TAGS. 18 are in addition to the serum and 51 more in mackerel. Finally, the distribution of fatty acids shows a high concentration of omega 6 and saturated fatty acid in serum and a high abundance of omega 3 and 9 in mackerel.

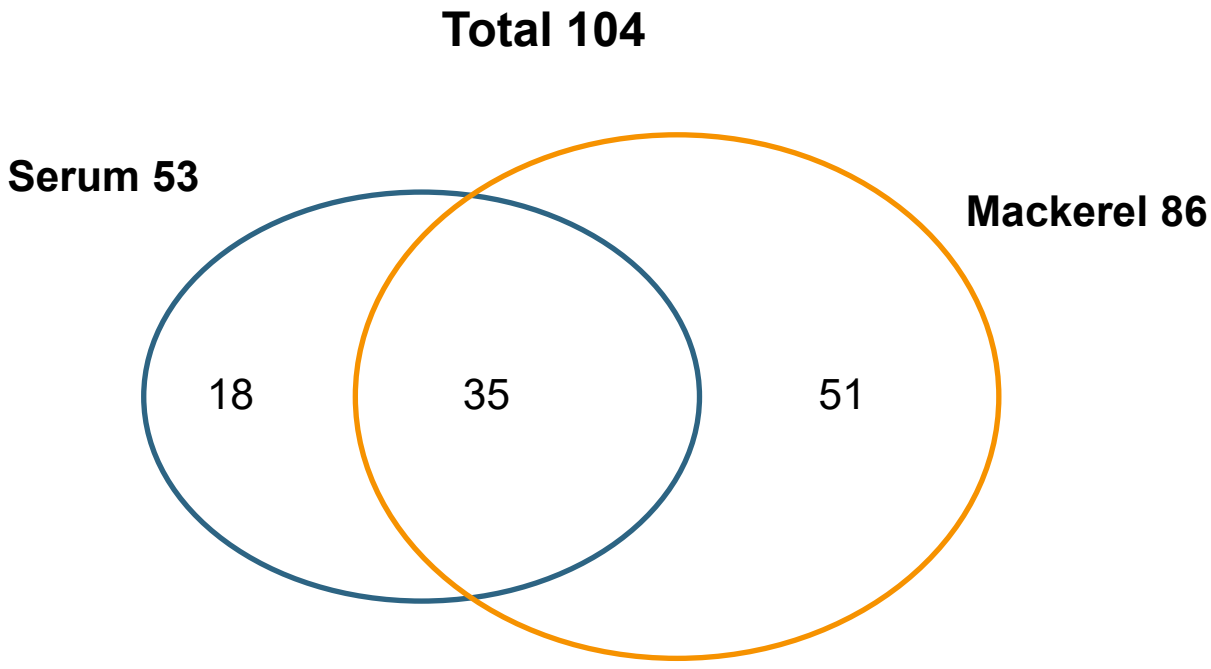


Figure 5 Venn Diagram for TAGs and Fatty Acids in Serum and Mackerel

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